A COMPARATIVE STUDY OF THE POPULATION DENSITIES OF VARIOUS TARDIGRADA SPECIES

An abstract of a Thesis by
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Drake University

The problem. The purpose of this investigation was to compare the population density of tardigrades in regions as related to: (a) high and low particulate counts without reference to the nature of the particulate present and (b) high and low traffic flow density without reference to particulate counts.

Procedure. Bark-lichen samples were collected from 12 species of deciduous trees from 12 locations in Polk County, Iowa. The regions varied in particulate count, measured in tons of dust fall per month, and traffic flow density, measured in number of cars per year. Tardigrades were collected from samples, mounted in lactophenol, and identified. The area of bark-lichen samples was measured and the densities calculated in number of tardigrades per square centimeter.

Findings. It appears from this study that tardigrades are sensitive to high amounts of dust fall and high amounts of automobile exhaust.

Conclusions. There appears to be a direct relationship between tardigrade population density and particulate count and traffic flow density. The higher the particulate count the lower the population density of tardigrades. The higher the traffic flow density the lower the tardigrade population density.

Recommendations. More work needs to be done in this area. There are many variables to take into consideration. Tardigrades may require certain conditions for growth which were not accounted for in this study. More needs to be learned about the tardigrade itself before this study becomes meaningful.

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A COMPARATIVE STUDY OF THE POPULATION DENSITIES OF VARIOUS TARDIGRADA SPECIES

A Thesis

Presented to

The School of Graduate Studies

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In Partial Fulfillment

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by

Cheryl Marie Baldree

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by

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INTRODUCTION

Tardigrades have been studied extensively in Europe, but have been largely neglected in the United States and Canada. Out of approximately 340 species described (Pennak, 1953) only 36 species have been reported from the United States (Riggin, 1964) and only about 40 species have been reported from Canada (Argue, 1971). Most tardigrade species have been described by persons who are not specialists in taxonomy and there is much confusion with their classification (Riggin, 1962).

Tardigrades are aquatic or semiaquatic animals. They may be found in ponds or marine environments, but the majority of species are associated with lichens, liverworts, and mosses (Pennak, 1953).

Tardigrades associated with lichens, liverworts, and mosses, unlike most marine and freshwater species, can resort to an inactive state called anabiosis. With the dessication of the habitat the animals lose water from the body cavity and retract their head, legs, and posterior portion. Metabolism is slowed down and the organism depends upon stored food for survival (Pennak, 1953).

Anabiotic tardigrades are resistant to extreme temperatures. Mathews (1938) reported they could tolerate temperatures of -200°C. for seven months without harm. Pennak (1953) reported that tardigrades subjected to -190°C. for twenty months survived. Tardigrades do not withstand

high temperatures as well, although they have been able to survive after being subjected to a temperature of 96°C. for one-half hour. Atmospheric pressure and brine solutions do not seem to affect them (Clothier, 1963). Radiation and high frequency sound waves do not seem to influence these tardigrades (Mathews, 1938). Gases such as carbon dioxide, helium, nitrogen, hydrogen, and hydrogen sulfide do not kill them. Sulfur dioxide gas, a common air pollutant, kills them quickly (Clothier, 1963).

It has been noted that air pollutants have a definite affect upon lichens and perhaps arthropod populations (Skye, 1968). Air pollutants such as sulfur fumes from coal, ozonated hydrocarbons from cooking gas and automobile exhaust, and high heat from city atmosphere affect lichen growth. Lichens tend to concentrate these toxic elements in their thallus (Hale, 1961). Tardigrade species which inhabit lichens growing on deciduous trees apparently have a high sensitivity to toxic elements.

This study is an attempt to determine whether differences can be shown in the population density of tardigrades in areas where high particulate counts have been recorded in the vicinity of Des Moines, and to compare population densities near street intersections with high traffic flow with those seen in rural areas.

MATERIALS AND METHODS

A total of 160 bark and lichen samples were collected between June 22, 1971 and July 30, 1971. They were taken from twelve species of deciduous trees between a height of one and two meters. Samples were selected so as to obtain a single lichen species. This was done to permit more precise comparisons and to reduce variability. The tree species and lichen species encountered are listed in Tables 1 and 2 respectively. The samples were obtained from twelve locations in Polk County, Iowa which are listed in Table 3. The sites from which the samples were taken were chosen to permit a comparison of tardigrade population density in areas with differences in particulate air pollution and in areas adjacent to street intersections with relatively high and low traffic loads.

The criteria used for site selection were particulate count and traffic flow density. Regions with high or low particulate counts were selected with the aid of data obtained from the Des Moines Health and Sanitation Department. The agency records particulate counts in tons of dust fall per month for various regions in Des Moines and neighboring areas. Table 3 lists the data recorded. High particulate counts in Iowa may be caused by agricultural practices such as plowing. The agricultural land containing bare soil along with wind may be responsible for dust. Emissions from industrial

TABLE 1. Deciduous tree species from which bark-lichen samples were taken

Quercus bicolor	*Quercus macrocarpa*
Acer barbatum	*Acer saccharinum*
Populus deltoides	*Acer platanoides*
Ulmus americana	*Celtis occidentalis*
Ulmus pumila	*Gleditsia triacanthos*
Fraxinus pennsylvanica lanceolata	*Morus alba*

TABLE 2. Tree-borne lichen species from which tardigrades were extracted

Candelaria concolor

Physcia orbicularis

Physcia stellaris

Parmelia rudecta

Xanthoria candelaria

TABLE 3. Regions studied in Polk County, Iowa with respect to particulate count and traffic flow density

Site	Particulate count (dust fall in tons/ month)	Traffic flow density (cars/year)
Polk City	21.4	----
Riverview Park	26.0	----
Clive	22.7	----
Urbandale	11.4	----
S.W. 9th & Leland (Army Post Road)	----	23,046
MacRae Park	----	----
S.E. 23rd & Maury	20 - 25	30,503
Capitol grounds		
E. 14th & Grand	20 - 25	25,440
E. 15th & Grand	20 - 25	26,374
S.W. 9th & Porter	----	17,498
Ewing Park (S.E. 14th & McKinley)	----	18,894
E. 1st & Des Moines St.	20 - 25	----
10th & Railroad	39.0	----

plants and motor vehicles may also contribute. Therefore, in rural areas, a high particulate count may not mean that a high concentration of toxic compounds is present. On the other hand, emissions from internal combustion engines are known to have a high concentration of toxic pollutants, even though the particulate count may be relatively low. Air monitoring in the Des Moines area has been mostly confined to particulate counts. To date, the toxicity of the particulate matter has not been determined. To determine the effect of toxic air pollutants on tardigrade populations, samples were taken in the region of street intersections known to have high traffic level and others known to have low traffic level. Traffic flow densities for street intersections, for the year 1970, were reported by the Des Moines Sunday Register, July 4, 1971. This information along with the data obtained from the Des Moines Health and Sanitation Department were used to select areas for sampling. See Table 3.

Summarizing then, the selection of sites for sampling was designed to permit the comparison of relatively high and low particulate counts on the tardigrade population. The study was made without reference to the nature of the particulate present. The sites were also chosen to permit, as an independent factor, the comparison of regions adjacent to intersections of relatively high traffic flow with those of relatively low traffic flow.

After collection, the bark-lichen samples were treated

in a routine fashion. The tardigrades were collected by placing each sample in an inverted position into separate plastic funnels. Each bark-lichen sample was totally immersed in spring water and weighted down with a syracuse watchglass for a twenty-four hour period. This procedure enabled the tardigrades to be released from the lichen substrate in an asphyctic state. Water containing the specimens was drained from the funnels and studied with a dissecting microscope. Tardigrades were extracted from the water using a finely drawn pipette. The specimens were immediately mounted on slides containing the preservative, lactophenol, which was developed by Bernhardt (1943). The slides served as an accurate account of the number of tardigrades found in each sample. They were also used for identification purposes of tardigrade species. The key used for identification was developed by G. Thomas Riggin Jr. (1962).

After the tardigrades were extracted from the spring water, the bark-lichen sample was rehydrated for a second twenty-four hour period. The second hydration insured collection of at least 95% of the tardigrades present in the sample (Kimmel, 1968). The water was drained and the tardigrades were collected as before. The bark-lichen samples were dried and saved.

To determine the percentage of lichen coverage, photographs were taken of each sample before hydration. The

photographs were superimposed on a grid of one-hundred evenly spaced points and the area of the total sample and lichen calculated. A tally of the points touching lichens (hits) and points on naked bark (misses) was made. The percentage of hits and misses approaches the percentage of lichen cover. This percentage was multiplied by the total area of the sample to provide an estimate of the area of lichen in the sample. The total number of tardigrades was stated per square centimeter of sample and lichen area.

Means, calculated for each set of samples, were based on the total number of tardigrades obtained from all samples and the total area of the samples, excluding the controls.

DATA OBTAINED

Data recorded from the deciduous trees in Polk County included the identification of several species of tardigrades. With the exception of Milnesium tardigradum and Hypsibius oberhaeuseri, the identification of other species is only tentative. The keys available vary greatly and there is much confusion below the genus level of classification. See Table 4.

Although not all species of tardigrades have the same sensitivity to air pollutants, recent work has shown little if any difference in sensitivity to sulfur dioxide or carbon monoxide (Michael Anderson, unpublished data). Therefore, only the total number of tardigrades are reported rather

than the number of individual species.

TABLE 4. Tardigrade species identified from tree-borne lichens in Polk County

Milnesium tardigradum	Macrobiotus richtersi
Macrobiotus hufelandi	Macrobiotus areolatus
Hypsibius oberhaeuseri	Macrobiotus tonollii
Macrobiotus echinogenitus	

The detailed account of the data obtained is found in tables in the Appendix which show the data recorded for each tree species in each location. The total sample area and lichen area are expressed in square centimeters. The total number of tardigrades per sample is given. The tardigrade population density is expressed as the number of tardigrades per square centimeter of sample area and as the number of tardigrades per square centimeter of lichen. The tables are first arranged according to particulate count. Subsequently tables are included where the dust fall is unknown with the traffic flow density. The tables list the regions with higher counts first, and then, proceed to the lower values. Table 5 summarizes the tables found in the Appendix.

TABLE 5. Summary of mean population densities taken from each tree and location

Tree	Location	Total sample area (cm^2)	Total lichen area (cm^2)	Density tard./ sample (cm^2)	Density tard./ lichen (cm^2)
Populus deltoides	10th & R.R.	41.98	27.63	0	0
Populus deltoides	Riverview	34.61	20.76	0.029	0.048
Quercus bicolor	Riverview	5.95	1.41	0	0
Gleditsia triacanthos	E. 1st & Des Moines	19.29	10.33	0	0
Gleditsia triacanthos	S.E. 23rd & Maury	60.48	20.94	0.364	1.051
Fraxinus pennsylvanica lanceolata	Capitol	18.15	5.26	0	0
Morus alba	Capitol	14.33	3.73	0.070	0.268
Acer saccharinum	Capitol	43.98	11.96	0.023	0.085
Acer platanoides	Capitol	13.63	7.64	0	0
Gleditsia triacanthos	Capitol	28.88	15.03	0	0
Ulmus pumila	Clive	41.18	25.78	0.486	0.776
Populus deltoides	Clive (parking lot)	18.73	5.77	0	0
Populus deltoides	Clive (woods)	22.83	12.20	5.081	9.508

TABLE 5. (continued)

Tree	Location	Total sample area (cm^2)	Total lichen area (cm^2)	Density tard./ sample (cm^2)	Density tard./ lichen (cm^2)
Acer barbatum	Polk City	77.08	63.72	1.271	1.698
Quercus bicolor	Polk City	80.39	60.20	2.227	2.977
Quercus macrocarpa	Urbandale (54th)	28.42	21.86	11.048	14.364
Quercus macrocarpa	Urbandale (65th)	13.14	8.89	0.076	0.112
Ulmus americana	Urbandale	33.06	29.10	4.023	4.570
Acer saccharinum	Urbandale	29.86	18.94	4.153	6.547
Acer saccharinum	S.W. 9th & Leland	22.31	11.41	0.762	1.490
Ulmus americana	S.W. 9th & Leland	28.63	21.00	0.035	0.048
Ulmus americana	Ewing Park	29.19	19.31	6.201	9.425
Celtis occidentalis	S.W. 9th & Porter	38.66	19.41	0.413	0.824
Quercus bicolor	MacRae Park	23.04	17.02	0.043	0.059
Gleditsia triacanthos	MacRae Park	24.20	19.37	0.909	1.136

DISCUSSION

Several factors may affect the habitat of tardigrades. The species of tree as well as the species of lichen may influence the population density of tardigrades. Physical factors may also affect the density. Such factors are light, heat, moisture, wind, and the height and direction of the sample site of the tree (Kimmel, 1968). In a study of this kind, such factors as these can not be controlled. Some randomization of these factors results from repetitive sampling, however.

Control samples which did not contain visible lichen cover were examined. See Tables 1-25 in the Appendix. In general, control samples from trees on which tardigrades were found contained some tardigrades, but tardigrades were less abundant in the absence of lichens. The surface of a tree is a very diversified habitat for an organism as small as a tardigrade. Variations occur in lichen and algal substrates, bark texture, and availability of moisture because of pathways along which rain normally drains off of the trunk. The presence of some tardigrades on bark not containing lichen means that the estimates of tardigrades per cm^2 of lichen surface are somewhat higher than they should be. The smaller population density of tardigrades on bark not containing lichens means that the estimates of tardigrades per cm^2 of total sample area are low, especially when lichen cover is

sparse. Both values are quoted in the tables reporting the results of this study. See the Appendix.

It is not yet clear to what extent the species of lichen and species of tree enter into the distribution of tardigrades, although both are factors (Kimmel, 1968). Before attempting to analyze the results with respect to the particulate count and the traffic flow density, it will be helpful to consider the results with respect to the biological substrate on which the tardigrades were living.

To provide a standard against which specific tree or lichen substrates can be measured, a mean for all samples was calculated. The average population density of tardigrades for all samples was 1.588 tardigrades per cm^2 of sample area and 2.628 tardigrades per cm^2 of lichen surface.

In order to interpret the significance of the tree species, the mean population density for each species was calculated and presented without reference to their position. See Table 6. Of the twelve species of trees studied only two failed to contain any tardigrades. Both of these species, Acer platanoides and Fraxinus pennsylvanica lanceolata, were located on the Capitol grounds where the particulate count and the traffic flow density were relatively high. This probably accounts for the lack of tardigrades present although it is not possible to make a definite conclusion since this was the only location from which samples were taken. The tree species with the lowest population density

TABLE 6. The mean population densities of each tree species

Tree species	Mean density tard./sample (cm^2)	Mean density tard./lichen (cm^2)
All samples	1.588	2.628
Quercus macrocarpa	7.579	10.244
Ulmus americana	3.477	4.538
Acer saccharinum	1.476	3.356
Acer barbatum	1.271	1.698
Quercus bicolor	1.646	2.289
Populus deltoides	0.990	1.894
Ulmus pumila	0.486	0.776
Celtis occidentalis	0.413	0.824
Gleditsia triacanthos	0.331	0.670
Morus alba	0.070	0.268
Acer platanoides	0	0
Fraxinus pennsylvanica lanceolata	0	0

of those having samples taken from several areas was Gleditsia triacanthos. The species showing the highest density was Quercus macrocarpa. Ulmus americana, Acer saccharinum, and Quercus bicolor had densities above the mean for all samples.

The locality of the tree as well as the tree species is important. See Table 7. There are several hypotheses which may be stated with regard to the tree species and location: (1) that different tree species can vary markedly even though their surroundings are rather similar; (2) that the same tree species can have quite different tardigrade densities if they have different surroundings; (3) and that since the same species of tree showed differing population densities when in different surroundings, the differences noted can not be wholly explained by differences in two species.

Populus deltoides illustrates the hypotheses well. Depending upon the amount of dust fall, the mean population densities vary markedly. It can be seen from Table 7 that Populus deltoides had a low mean density at Riverview Park, and Tenth and Railroad where the particulate count was relatively high. It is interesting to note the difference in densities in Clive. One of the trees was located in a post office parking lot, while the other was located in a wooded area. The tree located in the woods had a high mean population density while the one located in the parking lot

TABLE 7. A comparison of the mean population densities in the same tree species found in different areas

Tree species	Area	Dust fall tons/ month	Traffic flow cars/ year	Density tard./ sample (cm^2)	Density tard./ lichen (cm^2)
Populus deltoides					
	Clive(woods)	22.7	----	5.081	9.508
	Clive(post office)	22.7	----	0	0
	Riverview	26.0	----	0.029	0.048
	10th & R. R.	39.0	----	0	0
Acer saccharinum					
	Urbandale	11.4	----	4.153	6.547
	S.W. 9th & Leland	----	23,046	0.762	1.490
	Capitol	20-25	25,440-26,374	0.022	0.084
Ulmus americana					
	Urbandale	11.4	----	4.023	4.570
	S.W. 9th & Leland	----	23,046	0.035	0.048
	Ewing Park	----	18,894	6.201	9.425

TABLE 7. (continued)

Tree species	Area	Dust fall tons/ month	Traffic flow cars/ year	Density tard./ sample (cm^2)	Density tard./ lichen (cm^2)
Quercus macrocarpa					
	Urbandale (54th)	11.4	----	11.048	14.364
	Urbandale (65th)	11.4	----	0.076	0.112
Quercus bicolor					
	Polk City	21.4	----	2.227	2.977
	Riverview	26.0	----	0	0
	MacRae Park	----	----	0.043	0.059
Gleditsia triacanthos					
	MacRae Park	----	----	0.909	1.136
	E. 1st & Des Moines	20-25	----	0	0
	Capitol	20-25	25,440-26,374	0	0
	S.E. 23rd & Maury	20-25	30,503	0.364	1.051

had no tardigrades present. A possible explanation could be due to the high amount of automobile exhaust inflicted upon the tree.

In examining the mean densities for Acer saccharinum, it can be noted that there is a high population density in Urbandale which had a low dust fall of 11.4 tons/month. On the other hand, the trees at the Capitol grounds had a very low population density and the dust fall and traffic flow density was relatively high. The tree at S. W. 9th and Leland had a considerably higher density than the trees at the Capitol grounds. This could be accounted for by the fact that the traffic flow density was about 3,000 cars/year less.

Ulmus americana had a high population density in Urbandale where the particulate count was 11.4 tons/month. The density was also high at Ewing Park. This may be due to the fact that the tree was considerable distance from S. E. 14th and McKinley where the traffic flow density was measured at 18,894 cars/year. The density at S. W. 9th and Leland was very low, but the traffic flow density was around 4,000 cars/year greater.

Quercus bicolor showed a high population density in Polk City where the particulate count was 21.4. The same species in Riverview Park with a particulate count of 26 contained no tardigrades.

Quercus macrocarpa illustrates the first hypothesis. Even though both sets of samples were taken from Urbandale

where particulate count is low, the set of samples from 65th Street exhibited an extremely high mean population density while the set taken from 54th Street was very low.

In comparing mean densities, it appears that tardigrades do not favor Gleditsia triacanthos at any location, with the exception of MacRae Park for which no pollution data was gathered. The dust fall for E. 1st and Des Moines Street, the Capitol, and S. E. 23rd and Maury was between 20 and 25 tons of dust per month. The traffic flow for S. E. 23rd and Maury was about 5,000 cars higher per year than at the Capitol. The interaction of dust fall and automobile exhaust may account for the lack of tardigrades at the Capitol and the low density at S. E. 23rd and Maury. Since no accurate particulate count was made for those two regions, perhaps the dust fall was higher at the Capitol which may explain why no tardigrades were found there.

The lichen species need to be considered regardless of the tree species. The mean population densities of tardigrades for each lichen species and the three combinations of lichens found, was compared without reference to their position. See Table 8. Lichens may possess different characteristics which may hinder or enhance tardigrade growth. From the table it is apparent that the lichens, Parmelia rudecta, Xanthoria candelaria, and the combination of Parmelia rudecta and Candelaria concolor had the highest mean densities. Parmelia rudecta had by far the highest density which was

11.048 tardigrades per sample cm^2. There were no tardigrades found in the samples containing only Physcia orbicularis, but tardigrades were present when it was in combination with Candelaria concolor. Perhaps Physcia orbicularis is a poor substrate for tardigrades. With the exception of the tardigrade Hypsibius tuberculatus, Kimmel found P. orbicularis to be a poor substrate for tardigrades. P. stellaris in combination with C. concolor also shows a low population density.

TABLE 8. The mean population densities of each lichen species

Lichen species	Mean density tard./sample (cm^2)	Mean density tard./lichen (cm^2)
All samples	1.588	2.628
Parmelia rudecta	11.048	14.364
Xanthoria candelaria	6.201	9.425
Physcia stellaris	1.009	1.088
Physcia orbicularis	0	0
Parmelia rudecta & Candelaria concolor	4.153	6.547
Physcia orbicularis & Candelaria concolor	1.424	2.049
Physcia stellaris & Candelaria concolor	0.634	1.212

The locality of the lichen as well as the species is important. In order to interpret a relationship between the locality and the lichen species, a comparison was made of the mean population densities in each lichen species found in areas with high and low particulate counts, and high and low traffic flow density. See Table 9. It is also significant to note that in regions where the pollution level was high, it was difficult to find many lichens. P. rudecta and X. candelaria were not seen in areas with high particulate counts and traffic flow densities. This may be due to the fact that lichens have a high sensitivity to air pollutants (Skye, 1968). Since the sample is too small in this study, no real conclusion can be drawn.

Parmelia rudecta which was found only in Urbandale had a very high mean population density. Physcia orbicularis, on the other hand, found only in Clive, contained no tardigrades even though Clive had a relatively low dust fall of 22.7 tons/month. A possible reason for this may be the automobile exhaust in the post office parking lot.

Xanthoria candelaria, found only in Ewing Park, had a very high population density. The traffic flow was low, and the lichen was found a considerable distance from the street where the traffic flow density was recorded.

Physcia stellaris fluctuated, depending upon the pollution level. In areas where the particulate count was 25 tons per month or above, the mean population density

TABLE 9. A comparison of the mean population densities in the same lichen species found in different areas

Lichen species	Area	Dust fall (tons/ month)	Traffic flow (cars/ year)	Density tard./ sample (cm^2)	Density tard./ lichen (cm^2)
Parmelia rudecta					
	Urbandale	11.4	----	11.048	14.364
Physcia orbicularis					
	Clive	22.7	----	0	0
Xanthoria candelaria					
	Ewing Park	----	18,894	6.201	9.425
Physcia stellaris					
	10th & R. R.	39.0	----	0	0
	Riverview	26.0	----	0	0
	E. 1st & Des Moines St.	20-25	----	0	0
	Capitol	20-25	25,440-26,374	0	0
	Capitol	20-25	25,440-26,374	0.070	0.268
	Polk City	21.4	----	1.271	1.698
	Polk City	21.4	----	2.227	2.977
	S.W. 9th & Leland	----	23,046	0.762	1.490
	S.W. 9th & Porter	----	17,498	0.413	0.824

TABLE 9. (continued)

Lichen species	Area	Dust fall (tons/ month)	Traffic flow (cars/ year)	Density tard./ sample (cm^2)	Density tard./ lichen (cm^2)
P. stellaris & *C. concolor*					
	S.E. 23rd & Maury	20-25	30,503	0.364	1.051
	Capitol	20-25	25,440-26,374	0.022	0.084
	Capitol	20-25	25,440-26,374	0	0
	Capitol	20-25	25,440-26,374	0	0
	Clive	22.7	----	5.081	9.508
	Urbandale	11.4	----	0.076	0.112
	S.W. 9th & Leland	----	23,046	0.035	0.048
	MacRae Park	----	----	0.043	0.059
	MacRae Park	----	----	0.909	1.136
P. orbicularis & *C. concolor*					
	Riverview	26.0	----	0.029	0.048
	Clive	22.7	----	0.486	0.776
	Urbandale	11.4	----	4.023	4.570
P. rudecta & *C. concolor*					
	Urbandale	11.4	----	4.153	6.547

was zero or very minute. Polk City had a relatively low dust fall and the population density was highest there. The same is not true for the traffic flow density. Although the density at the Capitol is lower than it is at S. W. Ninth and Leland, it is greater at S. W. Ninth and Leland than it is at S. W. Ninth and Porter. S. W. Ninth and Leland had almost 6,000 more cars per year than S. W. Ninth and Porter.

Physcia stellaris and Candelaria concolor do not seem to be a suitable substrate for tardigrades in seven out of the nine areas in which they were found. Only in Clive where the dust fall was 22.7 tons per month did the density get way above the mean for all samples. In Urbandale with a dust fall of 11.4 tons per month, only a few tardigrades were found.

Physcia orbicularis and Candelaria concolor illustrated the hypothesis. In areas with a high particulate count the tardigrade population density is low. In areas with a low particulate count the population density is high.

Parmelia rudecta and Candelaria concolor were only found in Urbandale which had a dust fall of 11.4 tons per month. As before, the mean population density of tardigrades was high.

Since it is not possible to account for the differences in population density on the basis of lichen and tree species alone, Table 10 and 11 were established. Table 10 compares the mean population densities of tardigrades according to the

TABLE 10. A comparison of the mean population densities according to different particulate counts

Particulate count tons dust/month	Mean density tard./sample cm^2	Mean density tard./lichen cm^2
All samples	1.588	2.628
Dustfall over 25	0.012	0.020
Dustfall 20-25	1.018	1.843
Dustfall below 20	5.475	5.970

TABLE 11. A comparison of the mean population densities according to different traffic flow densities

Traffic flow density cars/year	Mean density tard./sample cm^2	Mean density tard./lichen cm^2
Mean of all samples	1.588	2.628
Traffic over 25,000	0.100	0.395
Traffic 20,000-25,000	0.353	0.555
Traffic below 20,000	2.903	5.088

different particulate counts. Table 11 compares the mean population densities of tardigrades according to the different traffic flow densities. It is apparent from the tables that the samples taken from areas with a dust fall of less than 20 tons per month had a higher mean density than those samples taken from an area with a dust fall greater than 20 tons per month. The same is true for the traffic flow density. The mean tardigrade population density is greater in those samples taken from an area with a traffic flow density greater than 20,000 cars per year. In general trees and lichens found in regions with high traffic flow densities and high particulate counts had lower mean tardigrade population densities than those found in regions of lower traffic flow and dust fall.

Although it is hard to reach a definite conclusion, it is possible that dust fall and traffic flow interact. Gleditsia triacanthos found at the Capitol grounds and at S. E. 23rd and Maury is the only instance in the data which suggests that dust fall and traffic flow may interact. The mean population density of tardigrades at the Capitol grounds is zero while the density at S. E. 23rd and Maury is 0.364 tardigrades per cm^2. The traffic flow at S. E. 23rd and Maury was also higher by about 5,000 cars per year. There was no data available from MacRae Park concerning dust fall or traffic flow, but one could assume that the traffic flow would be considerably less. The mean population densities

for that location is higher. It is possible that the interaction of the high traffic flow along with the high dust fall caused the low densities at the Capitol and S. E. 23rd and Maury.

Thus it has been shown that there is a direct relationship between the densities of tardigrade populations and areas with high and low pollution levels. Although the species of lichen and the species of tree have some influence on tardigrade population density, it has been shown that particulate matter and automobile exhaust are major controlling factors. In general, from this study, it has been shown that the higher the traffic flow density and the higher the particulate count, the lower the tardigrade population density.

SUMMARY

The purpose of this investigation was to compare the density of tardigrade populations in high and low regions as related to: (a) particulate counts without reference to the nature of the particulate present and (b) areas of high and low traffic flow density without reference to particulate counts.

A total of 160 bark-lichen samples were collected from twelve species of deciduous trees from twelve locations in Polk County. The regions varied in the amount of dust fall and automobile exhaust. Tardigrades were collected from the samples, mounted in lactophenol, and identified. The

areas of the bark-lichen samples were measured and the densities calculated in tardigrades per square centimeter.

From the data obtained it appears that tardigrades have a higher population density in those regions with a low particulate count and a low traffic flow density. Tardigrade sensitivity to sulfur dioxide and carbon monoxide may be involved in the low tardigrade population density in regions with high particulate count and high traffic flow density. Since there are so many factors involved, however, much more research needs to be done.

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APPENDIX

LIST OF TABLES

APPENDIX (continued)

APPENDIX (continued)

TABLE 1. Data obtained from *Physcia stellaris* on *Populus deltoides* at 10th & R. R.; dust fall 39 tons/month

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	2.98	0.00	0	0	0
153	3.32	1.82	0	0	0
154	6.63	3.24	0	0	0
155	8.62	7.35	0	0	0
156	5.67	1.35	0	0	0
157	3.74	3.53	0	0	0
158	5.45	5.04	0	0	0
control	3.13	0.00	0	0	0
19	3.40	2.24	0	0	0
20	5.15	3.06	0	0	0
mean density				0	0

TABLE 2. Data obtained from *Physcia stellaris* on *Quercus bicolor* at Riverview Park; dust fall 26 tons/month

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
Control	3.94	0.00	0	0	0
22	5.95	1.41	0	0	0
mean density				0	0

TABLE 3. Data obtained from Physcia orbicularis and Candelaria concolor on Populus deltoides at Riverview Park; dust fall 26 tons/month

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	7.58	0.00	0.00	0	0	0
16	4.54	3.50	0.71	0	0	0
17	9.15	3.95	1.75	1	0.109	0.175
18	4.23	1.52	0.08	0	0	0
60	4.13	1.91	0.44	0	0	0
61	2.44	1.34	0.00	0	0	0
62	4.24	1.33	0.75	0	0	0
63	5.88	2.64	0.84	0	0	0
mean density					0.029	0.048

TABLE 4. Data obtained from Physcia stellaris on Gleditsia triacanthos at E. 1st & Des Moines St.; dust fall 20-25 tons/month

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	2.23	0.00	0	0	0
145	6.73	2.58	0	0	0
147	2.07	1.63	0	0	0
148	1.72	1.45	0	0	0
149	5.24	1.88	0	0	0
150	1.85	1.42	0	0	0
151	1.68	1.37	0	0	0
mean density				0	0

TABLE 5. Data obtained from *Candelaria concolor* and *Physcia stellaris* on *Gleditsia triacanthos* at S. E. 23rd & Maury; dust fall 20-25 tons/month; traffic flow 30,503 cars/year

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	1.92	0.00	0.00	0	0	0
83	10.27	2.54	0.25	0	0	0
84	11.63	2.52	0.00	5	0.430	1.984
85	15.52	5.43	0.00	4	0.357	0.737
86	7.48	5.85	0.00	10	1.336	1.709
87	8.25	2.47	0.00	2	0.242	0.809
88	7.33	1.88	0.00	1	0.136	0.532
mean density					0.364	1.051

TABLE 6. Data obtained from *Physcia stellaris* on *Morus alba* at the Capitol Grounds; dust fall 20-25 tons/month; traffic flow 25,440-26,374 cars/year

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	2.52	0.00	0	0	0
124	7.58	2.63	0	0	0
126	1.83	0.48	1	0.546	2.083
127	4.92	0.62	0	0	0
mean density				0.070	0.268

TABLE 7. Data obtained from Physcia stellaris on Fraxinus pennsylvanica lanceolata at the Capitol; dust fall 20-25 tons/month; traffic 25,440-26,374 cars/year

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	2.83	0.00	0	0	0
120	2.95	1.88	0	0	0
122	4.41	1.69	0	0	0
123	10.79	1.69	0	0	0
mean density				0	0

TABLE 8. Data obtained from Physcia stellaris and Candelaria concolor on Acer saccharinum at the Capitol; dust fall 20-25 tons/month; traffic 25,440-26,374 cars/year

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	6.05	0.00	0.00	0	0	0
129	5.63	2.23	0.00	1	0.177	0.448
130	3.62	1.45	0.00	0	0	0
131	2.83	1.17	0.00	0	0	0
132	3.62	0.95	0.00	0	0	0
133	11.52	3.42	0.00	0	0	0
control	10.58	0.00	0.00	0	0	0
135	3.33	0.85	0.00	0	0	0
136	2.25	0.29	0.00	0	0	0
137	9.62	0.83	0.00	0	0	0
138	1.56	0.55	0.22	0	0	0
mean density					0.023	0.085

TABLE 9. Data obtained from *Physcia stellaris* and *Candelaria concolor* on *Acer platanoides* at the Capitol; dust fall 20-25 tons/month; traffic 25,440-26,374 cars/year

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	4.52	0.00	0.00	0	0	0
140	4.10	2.72	0.00	0	0	0
141	1.32	0.25	0.00	0	0	0
142	2.73	1.93	0.00	0	0	0
143	2.42	0.78	0.19	0	0	0
144	3.06	1.77	0.00	0	0	0
mean density					0	0

TABLE 10. Data obtained from *Physcia stellaris* and *Candelaria concolor* on *Gleditsia tricanthos* at the Capitol; dust fall 20-25 tons/month; traffic 25,440-26,374 cars/year

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	2.33	0.00	0.00	1	0.429	0
90	7.50	3.52	0.00	0	0	0
91	7.43	5.93	0.00	0	0	0
92	6.24	2.83	0.00	0	0	0
93	5.13	1.08	0.00	0	0	0
94	2.58	1.17	0.50	0	0	0
mean density					0	0

TABLE 11. Data obtained from Physcia orbicularis on Populus deltoides in Clive (parking lot); dust fall 22.7 tons/month

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	15.04	0.00	0	0	0
25	6.25	2.25	0	0	0
26	12.48	3.52	0	0	0
mean density				0	0

TABLE 12. Data obtained from Physcia orbicularis and Candelaria concolor on Ulmus pumila in Clive; dust fall 22.7 tons/month

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	6.89	0.00	0.00	2	0.290	1.000
29	4.78	3.63	0.00	6	1.255	1.653
30	8.17	5.56	0.00	2	0.245	0.359
31	5.15	3.64	0.33	0	0	0
32	5.42	2.33	0.00	1	0.184	0.429
33	7.75	3.27	0.43	1	0.129	0.306
34	6.08	2.84	0.00	5	0.822	1.761
35	3.83	3.75	0.00	5	1.310	1.330
mean density					0.486	0.776

TABLE 13. Data obtained from Candelaria concolor and Physcia stellaris on Populus deltoides in Clive (wooded lot); dust fall 22.7 tons/month

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	6.08	0.00	0.00	2	0.329	0
37	4.44	2.38	0.00	11	2.477	4.622
38	7.33	3.63	0.25	31	4.230	7.989
39	11.06	5.88	0.06	74	6.691	12.458
mean density					5.081	9.508

TABLE 14. Data obtained from Physcia stellaris on Acer barbatum at Polk City; dust fall 21.4 tons/month

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	3.15	0.00	5	1.587	0
1	25.64	22.94	21	0.819	0.915
2	14.05	9.89	15	1.068	1.516
3	5.06	3.88	20	3.953	5.154
4	6.75	4.76	20	2.961	4.201
5	5.38	4.47	29	5.371	6.487
6	7.35	6.24	2	0.272	0.320
7	12.85	11.54	1	0.078	0.087
mean density				1.271	1.698

TABLE 15. Data obtained from *Physcia stellaris* on *Quercus bicolor* at Polk City; dust fall 21.4 tons/month

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	2.68	0.00	5	1.862	0
8	23.75	17.94	3	0.126	0.167
9	8.54	2.85	15	1.756	5.264
10	6.85	5.94	52	7.598	8.754
11	6.54	5.39	8	1.223	1.484
12	6.35	5.75	25	3.937	4.347
13	9.46	6.98	5	0.529	0.716
14	10.64	7.84	71	6.673	9.056
15	8.26	7.51	0	0	0
mean density				2.227	2.977

TABLE 16. Data obtained from *Parmelia rudecta* on *Quercus macrocarpa* in Urbandale; dust fall 11.4 tons/month

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	5.23	0.00	0	0	0
40	11.38	7.13	141	12.390	19.775
41	9.83	8.02	81	8.240	10.099
42	7.21	6.71	92	12.760	13.711
mean density				11.048	14.364

TABLE 17. Data obtained from *Physcia stellaris* and *Candelaria concolor* on *Quercus macrocarpa* in Urbandale (65th St.); dust fall 11.4 tons/month

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	2.94	0.00	0.00	0	0	0
44	5.25	4.65	0.00	0	0	0
45	3.38	1.18	0.43	1	0.190	0.215
46	4.51	2.63	0.00	0	0	0
mean density					0.076	0.112

TABLE 18. Data obtained from *Parmelia rudecta* and *Candelaria concolor* on *Acer saccharinum* in Urbandale; dust fall 11.4 tons/month

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	3.25	0.00	0.00	15	4.615	0
53	5.69	4.44	0.00	4	0.703	0.901
54	9.17	0.54	2.18	36	3.926	13.235
55	5.85	0.00	4.48	0	0	0
56	3.58	2.64	0.00	12	3.352	4.545
57	3.06	2.48	0.00	9	2.941	3.629
58	2.51	2.18	0.00	63	25.098	28.899
mean density					4.153	6.547

TABLE 19. Data obtained from *Physcia orbicularis* and *Candelaria concolor* on *Ulmus americana* in Urbandale; dust fall 11.4 tons/month

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	2.56	0.00	0.00	0	0	0
48	22.75	18.89	1.93	84	3.692	4.037
49	4.05	2.72	0.32	6	1.481	1.974
51	2.63	2.49	0.00	5	1.901	2.008
52	3.63	1.92	0.83	38	10.468	13.818
mean density					4.023	4.570

TABLE 20. Data obtained from *Candelaria concolor* and *Physcia stellaris* on *Ulmus americana* at S. W. 9th & Leland; traffic flow 23,046 cars/year

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	3.08	0.00	0.00	0	0	0
64	6.24	4.34	0.00	1	0.160	0.230
65	1.81	0.16	1.24	0	0	0
66	8.88	7.13	0.00	0	0	0
67	7.44	5.46	0.23	0	0	0
68	1.94	0.21	1.15	0	0	0
69	2.32	0.00	1.08	0	0	0
mean density					0.035	0.048

TABLE 21. Data obtained from *Physcia stellaris* on *Acer saccharinum* at S. W. 9th & Leland; traffic flow 23,046 cars/year

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	7.75	0.00	1	0.129	0
102	2.59	2.34	0	0	0
103	8.13	1.35	9	1.110	6.667
104	3.95	1.08	0	0	0
105	3.09	2.59	2	0.649	0.772
106	4.55	4.05	6	1.310	1.481
mean density				0.762	1.490

TABLE 22. Data obtained from *Xanthoria candelaria* on *Ulmus americana* at Ewing Park (S. E. 14th & McKinley); traffic flow 18,894 cars/year

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	5.67	0.00	22	3.880	0
108	1.83	0.85	2	1.090	2.342
109	1.50	0.88	6	4.000	6.857
110	2.58	2.13	2	0.665	0.939
111	6.95	3.35	93	13.381	27.761
112	7.85	6.37	41	5.235	6.436
113	8.48	5.73	37	4.362	6.457
mean density				6.201	9.425

TABLE 23. Data obtained from *Physcia stellaris* on *Celtis occidentalis* at S. W. 9th & Porter; traffic flow 17,498 cars/year

Sample number	Sample area cm^2	Lichen area cm^2	Tard.	Density tard./sample cm^2	Density tard./lichen cm^2
control	6.06	0.00	3	0.495	0
96	13.58	10.43	7	0.515	0.661
97	8.75	2.91	1	0.114	0.344
98	9.79	4.38	1	0.102	0.228
99	6.54	1.69	7	1.070	4.142
mean density				0.413	0.824

TABLE 24. Data obtained from *Physcia stellaris* and *Candelaria concolor* on *Quercus bicolor* at MacRae Park; no data available

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
control	2.28	0.00	0.00	0	0	0
71	3.95	3.22	0.08	0	0	0
72	4.62	2.45	0.00	0	0	0
73	6.43	4.63	0.65	0	0	0
74	4.90	2.77	0.88	1	0.204	0.862
75	3.14	2.34	0.00	0	0	0
mean density					0.043	0.059

TABLE 25. Data obtained from *Physcia stellaris* and *Candelaria concolor* on *Gleditsia triacanthos* at MacRae Park; no data available

Sample number	Sample area cm^2	Lichen area 1 cm^2	Lichen area 2 cm^2	Tard.	Density tard./ sample cm^2	Density tard./ lichen cm^2
Control	2.81	0.00	0.00	0	0	0
78	2.58	1.53	0.00	0	0	0
79	4.69	4.51	0.00	8	1.705	1.774
80	4.80	3.42	0.00	2	0.416	0.585
81	3.38	1.45	0.88	0	0	0
82	8.75	7.58	0.00	12	1.370	1.583
mean density					0.909	1.136